Table 2

Effect of tPA Signal Sequence or Secretion of TNFR1-IgG1 Glycosylation Mutants

Mutant	% Secretion Efficiency* TNFR.ss	tPA.ss	EC50 for TNF Binding‡ (nM)
NNNN	50	70	6.66 +/- 0.73
NNQQ	20	70	4.74 +/- 0.48
NSNQ	<5	60	6.94 +/- 0.83
NKKQ	10	40	ND
QSNQ	<5	65	2.34 +/- 0.22
QQQQ	<5	<5	ND

* Data represent results from pulse-chase experiments (e.g. Figure 3) where the percentage of pulse-labeled protein secreted in a 24 hr period was measured by scanning densitometry.

‡Data represent EC50 values for the displacement of (^{125}I)-labeled TNF by TNFR-IgG1 glycosylation site mutants (Figure 7).

For example, only 10-20 % of the NNQQ mutant was secreted using the TNFR signal sequence, whereas 60-70% of the NNQQ mutant containing the tPA signal/pro sequence was secreted (Figures 3C and D, Figure 5). Similarly, little or none of the QSNQ mutant was secreted containing the TNFR signal sequence, but approximately 60% was secreted from the tPA signal/pro sequence containing variant (Figures 3E and F, Figure 5). Similar improvements in secretion efficiency were observed for the NKKQ and NSNQ mutants (Figure 5). Densitometric analysis of pulse chase experiments (Figure 3) showed that attachment of the tPA signal/pro sequence accelerated the kinetics of intracellular transport as well as increasing the total amount of secreted protein. Although replacement of the tPA /pro sequence could overcome the blockade of protein secretion for many of the glycosylation mutants, this strategy was not effective for the fully glycosylated QQQQ glycosylation site mutant (Figure 5).